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Distinct adsorption kinetics of Qβ and GA bacteriophages on drinking water biofilms

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Abstract The data of adsorption kinetics of F-specific RNA bacteriophages Qβ and GA on drinking water biofilms under hydrostatic conditions was modeled. The rate limitation of virus adsorption was shown to be the free diffusion in water for GA where as another rate limiting step was demonstrated for Qβ. Modeling results also showed that the number of adsorbed viruses can be fitted with a limitless equation in static conditions. However sorption–desorption assays carried out in dynamic conditions showed that Qβ and GA phages have a similar affinity for the biofilm and reinforced that no significant virus desorption occurred during the first 10 h of adsorption from a bulk containing virus in static conditions. The small surface properties variations between the two phages do not induce significant differences of their adsorbed quantities in hydrodynamic conditions but they significantly affect the rate at which adsorption occurs in hydrostatic conditions.

Keywords Bacteriophage adsorption · Adsorption kinetics modeling · Drinking water · Biofilm

Abbreviations

A	Surface area of the reactor in contact with water
D	Diffusion coefficient of the viruses in water
HDPE	High density polyethylene
K_{ads}	Adsorption constant
N_{ads}	Number of viruses adsorbed on the overall surface area of the reactor
$N_{ads.eq}$	Number of viruses adsorbed on the surface area of the reactor at equilibrium
$\tilde{N}_{ads}$	Number of viruses adsorbed normalized by the N_0, V and A
N_0	Number of viruses introduced in the system
PFU	Plaque Forming Units
t	Time
V	Overall volume of the reactor
α_0	Statistic factor taking into account the efficiency of the contact between the viruses and the surface
β	Characteristic time of site occupancy
δ_0	Distance parameter allowing potential initial adsorption (at experimental $t = 0$) to be taken into account
δ_1	Diffusion layer thickness parameter allowing a potential kinetic limitation of the flux to be taken into account

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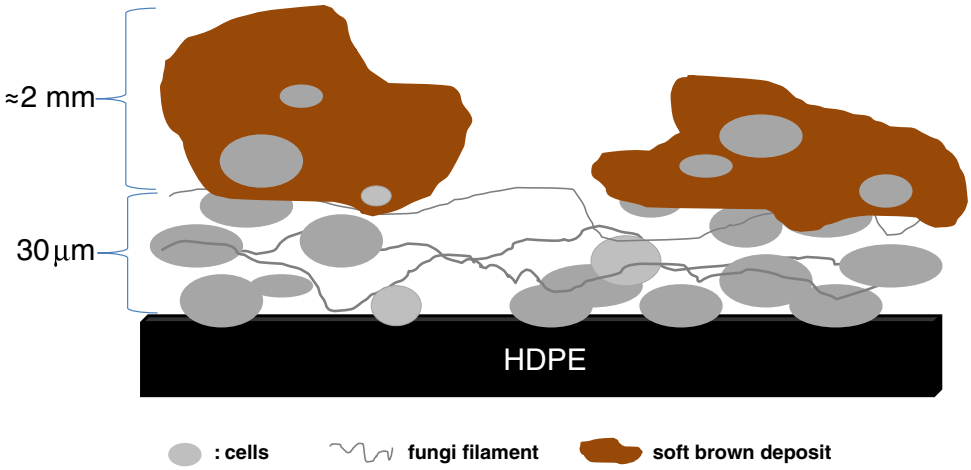

Fig. 1 Schematic view of a drinking water biofilm on HDPE (High Density PolyEthylene) pipe walls

1 Introduction

The accumulation of enteric viruses and bacteriophages (used as models) on drinking water biofilms has been previously documented at lab scale in static systems and in pilot mimicking pipes under flow (Quignon et al. 1997; Helmi et al. 2008; Pelleïeux et al. 2012). Such experimental works can be useful in predicting the transport of the viruses all along drinking water networks. However, it requires the assessment of rates at which adsorption to and desorption from the biofilm occur. Drinking water biofilms (Fig. 1) behave as viscoelastic systems thanks to their exopolymer matrix (Abe et al. 2011, 2012; Mathieu et al. 2014) and they represent sticky materials, which can trap colloidal particles. As a result, biofilms in drinking water systems act as environmental reservoirs for pathogenic microorganisms (Wingender and Flemming 2011).

Drinking water biofilms represent a complex biophysical world (bacterial cells and filamentous fungi combined with organics and minerals), patchy distributed on the material surface. They are embedded in an exopolymeric matrix (EPS), which cohesion is driven by hydrophobic and electrostatic interactions and multivalent cross-linking cations bridging negatively charged sites (Mathieu et al. 2014). As shown on Fig. 1 such fouling showed an heterogeneous distribution with soft brown deposits bearing iron oxides (up to few millimeters in high with approximately 10^6 bacteria/cm^2), and thin layers of cells sticked strongly to the substratum (30 µm thickness, 10^7 bacterial cells/cm^2 on average). Bacteriophages behave as soft colloidal particles (Langlet et al. 2008; Michen and Graule 2010; Dika et al. 2011), which can effectively adsorb on surfaces and on biofilms (Helmi et al. 2008; Storey and Ashbolt 2001; Pelleïeux et al. 2012). Due to their size, they are subjected to Brownian motion in the bulk but their kinetics of transfer from the water phase to the biofilm is poorly described. Recently we have reported the accumulation of F-specific RNA bacteriophages (Qβ and GA) on drinking water biofilms grown on HDPE (high density polyethylene) under non-flow conditions (Pelleïeux et al. 2012). We found GA and Qβ phages accumulate in different proportions on pipe walls and we hypothesized the different surface properties of these colloids could control the higher accumulation of GA phage. Here we tested different mathematical models to describe the adsorbed virus number versus time data. For modeling, two a priori assumptions were done: (1) the maximum number of adsorbed viruses is negligible compared to initial number N_0 injected in the water bulk, and (2) the bacteriophages are small colloidal particles (on average 26.3 nm in diameter) (Tars et al. 1997) subjected to Brownian thermal motion (Diffusion coefficient calculated by the Stokes–Einstein relation in water: $D = 1.67 \times 10^{-11}$ m^2 s^{-1}). In this work, we show that in spite of their relative similarities in terms of size (Langlet et al. 2008), the kinetics of adsorption of these two viruses in hydrostatic conditions do not obey the same physical model, indicating a different rate limiting step in the overall adsorption process. Additional experimental results obtained under hydrodynamic conditions revealed also that these different kinetic behaviors do not rest on a different affinity of the two phages for the biofilm.

2 Materials and methods

2.1 Materials

Biofilms were grown on HDPE coupons at 19 °C for two months in drinking water on a rotating disc, which speed was fixed at 75 rpm. The biofilm rotating disc reactor as well as the preparation of Qβ and GA bacteriophage suspensions and the quantification of their RNA genome by

real-time reverse transcription-PCR (real-time RT-PCR) have been previously described (Pelleïeux et al. 2012).

2.2 Modeling

For modeling we used some data provided by (Pelleïeux et al. 2012). Six mathematical models (described in the Supplementary data), which represent different combinations of mechanistic and mass transfer adsorption rate limitations, with or without a rapid adsorption equilibrium close to the interface, and with or without a limited number of adsorption sites were investigated in this work. The results of data fitting for each model and virus are shown in table S1.

2.3 Experimental set-up for adsorption assays

To perform analyses of virus accumulation kinetics on surfaces in hydrostatic conditions, the bacteriophages were spiked at the same time in the disc reactor filled with tap water (with no renewal during the experiment) at a final concentration of approximately 6 $\log_{10}$eq. PFU/mL (equivalent to Plaque Forming Unit/mL) for each phage. After mixing for 1 min, a 1 mL sample was collected to determine the initial concentration of the phages in water. Then the disc rotation was stopped (t = 0) and at appropriate time points (from 1 min to 10 h), two coupons and 1 ml water samples were collected simultaneously and analyzed in order to determine virus concentrations using real-time RT-PCR on surfaces and in water. Each coupon was dipped for 1 min in 20 mL of tap water to remove the not adhered phages. Two experiments were performed in the same conditions (i.e. n = 4). Additionally, the relative standard deviation calculated from the two independent sets of determinations and estimated for all measurements was equivalent to 31 %. To allow comparison between results, the crude N_{ads}/A values (N_{ads} is the number of adsorbed virus and A the area of adsorption) were systematically normalized taking into account the initial bulk concentration of virus $C_0 = N_0/V$ (N_0 the number of virus inoculated in the assay and V the bulk volume of the reactor). Thus, in the following the adsorption results are given as $\tilde{N}_{ads}$ defined as:

$$\tilde{N}_{ads} = \frac{N_{ads} V}{N_0 A} \quad (1)$$

2.4 Experimental set-up for desorption assays

Desorption assays were carried out in dynamic conditions (rotating disc speed: 75 rpm) for 3 h. First, phages (approximately 6 $\log_{10}$eq. PFU/mL for each phage) were spiked in the reactor with HDPE coupons colonized by a two-month-old biofilm and let adsorb for 3 hours (disc rotation speed: 75 rpm) (adsorption step). Then the disc with the coupons was transferred to another similar reactor containing tap water without phages and the disc was turning on at 75 rpm for three hours (desorption step). Adsorbed phages before and after the desorption assay were measured by real-time RT-PCR.

3 Results and discussion

3.1 Only diffusion in water limits GA phage adsorption onto biofilm

The immersion of biofilm coupons in a dispersed suspension of GA phage for 10 h at 20 °C under hydrostatic conditions showed a measurable sorption of these soft particles on the surface which attained a concentration $N_{ads}/A = 6 \times 10^4$ eq. PFU/cm^2. Among the 6 models tested here (see supplementary material), we only discuss the two simplest ones allowing fitting the data. Indeed at the same time elaborated models may fit the data and we cannot completely rule off the physical phenomena on which they are based but they do not improved significantly the data description compared to the simple models based on likely phenomena. The best fit, i.e. the one with the least adjustable parameter number and the least sum of the squared errors (Table S1 in Supplementary data), was obtained thanks to the B model. It is given by the Ilkovic equations in which D is the diffusion coefficient (Bard and Faulkner 2001):

$$N_{ads} = A \frac{2N_0}{V} \sqrt{\frac{D}{\pi} t} \quad (2)$$

In this model, the adsorption rate is determined only by diffusion in water toward a biofilm having a strong affinity for the virus (2).

The transfer and sorption of virus onto the biofilm-colonized surface in hydrostatic conditions is rapid since approximately 1,000 viruses can be detected after only 1 min of immersion in the suspension. It is noticeable that the full line, obtained with no adjustable parameter, indicates the maximum permitted by diffusion with no other kinetic barrier. The speed of sorption decreases over the time but even after 10 h the system did not reach equilibrium, moreover the first 10 h adsorption data can be fitted with a limitless equation. According to the model used to describe the data, no equilibrium may be obtained, such a trend being completely screened by logarithmic presentations often used in many works. The adsorption rate decrease is related to the increasing with time of the diffusion distance of the virus to reach the surface. Indeed the diffusion distance after 1 h of immersion in such static conditions can be as high as 500 μm (Figure S1 in Supplementary data).

The effective and strong adsorption of GA phage on biofilms should be related to its surface properties. They can be described under two main parameters: the charge density (which governs repulsive electrostatic forces with other negatively charged particles and surfaces), and especially the hydrophobicity (which allows strong interactions between hydrophobic domains). Indeed with much more bigger objects such as bacteria it was found that hydrophobicity was the main parameter controlling bacteria adhesion (Tatchou-Nyamsi-König et al. 2008). As a consequence, GA phage with its relatively low negative surface charge density and relatively high hydrophobicity could stick very efficiently to hydrophobic domains existing in biofilm exopolymeric matrix (Aldeek et al. 2013). As GA phage behavior is well described by the model B "diffusion in water", the adsorption appears to be irreversible in the 10 h range. It means also that in our conditions, the sorption of the phages on these potable drinking water biofilms grown at 19 °C is non site-specific and the phages are not internalized in the bacterial cell. Indeed these F-specific RNA phages cannot find attachment sites on the F-pili of their host bacteria (e.g. *E. coli*) which should not be found in potable waters and synthesized F-pili only for temperatures above 25 °C (Woody and Cliver 1995).

3.2 Qβ phage sorption is slower than expected from diffusion in water

As shown on Fig. 2, the sorption of Qβ on biofilm was less effective than GA. As the data are normalized, would the adsorption rate be limited only by diffusion in water it would be fitted by the same full line as GA. We tested the six models (Table S1), and we found that the pseudo-secondary order equation fits (model F) as well as the exponential equation (model A). Bearing in mind that in this work a virus is accounted for as adsorbed as soon as it is in contact with the biofilm, an explanation based on the virus diffusion in complex matrix of the biofilm thus does not hold. The second order equation has been shown to take into account intraparticle diffusion or surface reaction limitation (Plasinski et al. 2013). Intraparticle diffusion is not relevant to our experiments and surface reaction is redundant in our experimental conditions by the A model. Fits with A and F models thus bring to same conclusion: there is indeed a mechanistic rate limiting step to adsorption of Qβ on biofilm since the diffusion of the viruses is much faster than their adsorption. The concentration gradient zone does not exist in this case, and the integrated rate of the adsorption reaction gives:

$$N_{ads} = N_{ads.eq}\left(1 - e^{-k_{obs}t}\right) \quad (3)$$

The expected curve has, in this case, a monoexponential shape. In this situation, the adjusted parameters have the following values: $k_{obs} = 4.95 \times 10^{-5}\ s^{-1}$, and $N_{ads.eq}/A = 8.0 \times 10^{4}$eq. PFU.cm^{-2} (Table S1 in Supplementary data gathers normalized data). Since it is easily calculated with Eq. 3 that $t > 5/k_{obs}$ implies that $N_{ads} > 0.99\ N_{ads.eq}$, we assume that $t = 5/k_{obs}$ is the time needed to reach equilibrium. It is noticeable that this $5/k_{obs}$ value is about 28 h, indicating that all the results (acquired at t = 10 h max) referred to a situation far from the expected equilibrium, rendering questionable the precision of the $N_{ads.eq}$ predicted value.

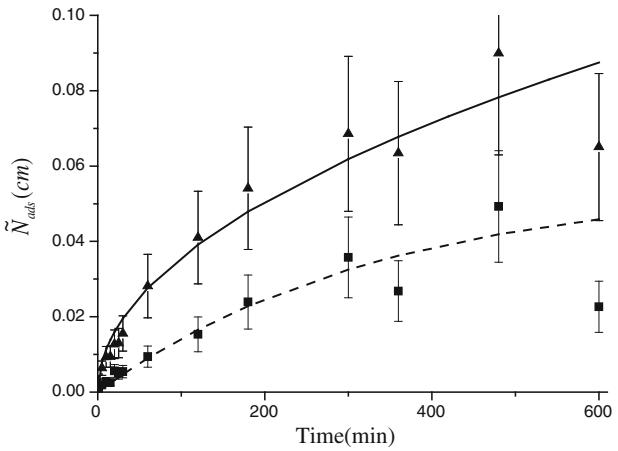

Fig. 2 Modeling of the adsorption of GA (filled triangle and full line) and Qβ (filled square and dashed line) under static conditions as a function of time. The full and dashed lines are respectively representative of the eq. $y = (1.25 \times 10^{-5}\ t)^{0.5}$ and $y = 0.0552\ \exp(2.95 \times 10^{-3}\ t)$ with t in min (the experimental data originate from Pelleïeux et al. 2012)

The unexpected result of our work is that the two phages (Qβ and GA) which have the same apparent diameter (approximately 26 nm) and the same diffusivity coefficient exhibit definitively different behaviors, showing no limitation of the sorption on biofilm for GA (but a pure mass transfer limitation), when on the contrary the rate of adsorption for Qβ was not controlled by its diffusion in water. Trying to go further in the understanding, we evaluated the impact of different values of the diffusion coefficient a priori estimated. Indeed the diffusion coefficient used for all the calculations came from the Stokes–Einstein relation for a sphere of 26.3 nm in diameter, which is the diameter of GA (Tars et al. 1997). Describing the experimental data of Qβ by means of the free diffusion model would require adjusting the diffusion coefficient at a value 5 times lower than the calculated. The diffusion coefficient value of well-dispersed virus suspension was experimentally determined in a previous work (Langlet et al. 2008) to be 1×10^{-7} cm^2 s^{-1} which is in excellent agreement with the value of 1.67×10^{-7} cm^2 s^{-1} considered in all our calculations. An explanation based on intrinsic Qβ diffusion properties in water thus does not stand.

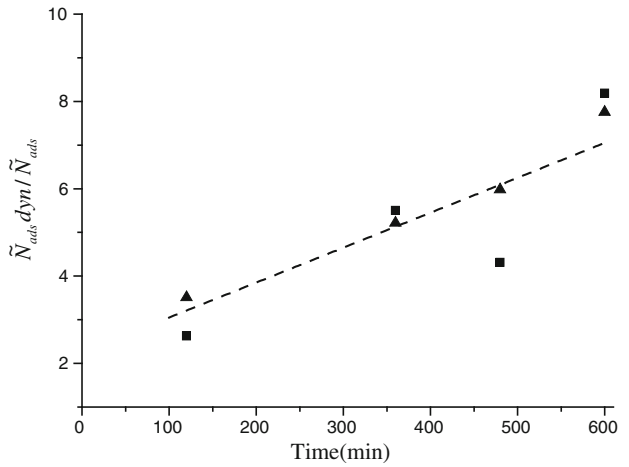

Fig. 3 Ratios $\tilde{N}_{ads}$ under hydrodynamic conditions (rotating disc at 75 rpm)/$\tilde{N}_{ads}$ under static conditions versus time (filled triangle GA phage, filled square Qβ phage)

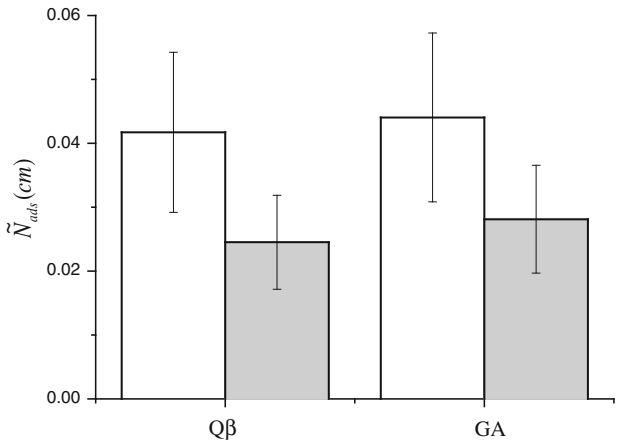

Fig. 4 Sorption on and desorption from drinking water biofilm of Qβ and GA phages under hydrodynamic conditions at 75 rpm in 3 h (sorption assay: *white bars*; desorption assay: *grey bars*)

When comparing the data calculated here from the data provided by Pelleïeux et al. (2012) in dynamic conditions with the one obtained in static conditions (Fig. 3), one may recognize that the number of adsorbed viruses is higher under dynamic conditions for the two viruses. This was expected for GA as one decreases the thickness of the diffusion gradient by stirring the system. Surprisingly, the same effect was observed for Qβ. Indeed the sorption kinetics model allowing to fit the experimental data excludes the existence of any concentration gradient zone, and thus there was no expected influence of the convection. A tentative explanation could be a modification of the biofilm surface and/or structure by the hydrodynamic stress. Without any evidence of such a modification on both the cells or the exopolymer matrix organization, trying to go further in the understanding is not possible at this stage.

3.3 Virus desorption from the biofilm is low

Our adsorption result analysis revealed that we only studied the early stages of the adsorption when we considered 10 h as the time range. Studying the adsorption on a broader time range is not possible since a significant biofilm evolution could occur during the experiments. We thus performed experiments in hydrodynamic conditions in 3 h, with a controlled stirring, with the particular aim of describing the viruses desorption. From the preceding sorption kinetic study, since GA adsorbs apparently in unlimited quantities on the biofilm, being trapped in its viscous matrix, at a rate only defined by its diffusion in water, one may forecast that a limited number of viruses will be released and independently of its released quantity, desorption will be slow. Inversely, regarding Qβ adsorption kinetics results, the modeling leads to a predicted $N_{ads.eq}$ value indicating that desorption occurs, at least at a long time scale. The sorption data reported in Fig. 4(white bars) indicates that in the shear rate conditions applied here, GA and Qβ phages exhibited (after 3 h) the same apparent affinity for the biofilm. It is indicating that the applied stirring allowed to overpass the rate limitation adsorption step for GA and seemed to change the rate limitation step for Qβ (which was already observed in previous experiments as discussed earlier in the text). After 3 h of phage desorption, more than 50 % of both pre-adsorbed viruses were still sorbed to the biofilm. This confirms that both viruses exhibit the same behavior towards the biofilm in hydrodynamic conditions. The fact that less than 50 % viruses desorbed when the contaminated biofilm is put into contact with clean water under stirring reinforces the view coming out from modeling that no significant desorption (i.e. number of virus desorbing much lower than the experimental uncertainty) occurred during our adsorption experiments in static conditions.

To summarize, in our quest for understanding the interactions of GA and Qβ phages with drinking water surface materials, we performed modeling of experimental data to describe the kinetics of their adsorption in hydrostatic conditions. Among the six models tested only the two simplest were discussed, indeed owing to the experimental uncertainty more elaborated model are not improving significantly the data description. Despite the experimental uncertainty inherent to the quantitative determination of adsorbed viruses, after modeling we may assert that GA adsorption is only limited by its diffusion in water. GA and Qβ phages appeared to have both a strong affinity for the biofilm, which can host them in great quantities but at different rates. The virus desorption from the biofilm or

virus release associated to biofilm detachment happens to be not significant within the time range of our experiments, even if it may not be excluded on longer time scales. The modeling data reported in this work revealed differences in adsorption rates of GA and Qβ phages.

This was not predictable on the basis of the previously reported surface physicochemical properties of these two phages (Langlet et al. 2008). Indeed, the surface hydrophobicity which could be related to the phage affinity for the biofilm is slightly higher for GA than for Qβ and there is no significant difference in the amount that would be adsorbed at equilibrium. Moreover the surface charge density, which is potentially leading to electrostatic repulsion when the phage approaches the biofilm and thus may modify the rate at which the adsorption occurs, is almost the same for the two phages. In other words the small surface properties variations between the two phages do not induce significant differences affinity for the biofilm as illustrated by adsorbed quantities in hydrodynamic conditions but they significantly affect the rate at which adsorption occurs in hydrostatic conditions.

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References

Abe, Y., Polyakov, P., Skali-Lami, S., Francius, G.: Elasticity and physico-chemical properties during drinking water biofilm formation. Biofouling **27**, 739 (2011)

Abe, Y., Skali-Lami, S., Block, J.-C., Francius, G.: Cohesiveness and hydrodynamic properties of young drinking water biofilms. Water Res. **46**, 1155 (2012)

Aldeek, F., Schneider, R., Fontaine-Aupart, M.-P., Mustin, C., Lécart, S., Merlin, C., Block, J.-C.: Patterned hydrophobic domains in the exopolymer matrix of *Shewanella oneidensis* MR-1 biofilms. Appl. Environ. Microbiol. **79**, 1400 (2013)

Bard, A.J., Faulkner, L.R.: Electrochemical Methods. Fundamental and Applications, 2nd edn. Wiley, New York (2001)

Dika, C., Duval, J.F.L., Ly-Chatain, H.M., Merlin, C., Gantzer, C.: Impact of internal RNA on aggregation and electrokinetics of viruses: comparison between MS2 phage and corresponding virus-like particles. Appl. Environ. Microbiol. **77**, 4939 (2011)

Helmi, K., Skraber, S., Gantzer, C., Willame, R., Hoffmann, L., Cauchie, H.M.: Interactions of *Cryptosporidium parvum*, *Giardia lamblia*, vaccinal poliovirus type 1, and bacteriophages phiX174 and MS2 with a drinking water biofilm and a wastewater biofilm. Appl. Environ. Microbiol. **74**, 2079 (2008)

Langlet, J., Gaboriaud, F., Duval, J.F., Gantzer, C.: Aggregation and surface properties of F-specific RNA phages: implication for membrane filtration processes. Water Res. **42**, 2769 (2008)

Mathieu, L., Bertrand, I., Abe, Y., Angel, E., Block, J.C., Skali-Lami, S., Francius, G.: Drinking water biofilm cohesiveness changes under chlorination or hydrodynamic stress. Water Res. **55**, 175 (2014)

Michen, B., Graule, T.: Isoelectric points of viruses. J. Appl. Microbiol. **109**, 388 (2010)

Pelleïeux, S., Bertrand, I., Skali-Lami, S., Mathieu, L., Francius, G., Gantzer, C.: Accumulation of MS2, GA, and Qβ phages on high density polyethylene (HDPE) and drinking water biofilms under flow/non-flow conditions. Water Res. **46**, 6574 (2012)

Plasinski, W., Dziuba, J., Rudzinski, W.: Modeling of sorption kinetics: the pseudo-second order equation and the sorbate intraparticle diffusivity. Adsorption **19**, 1055 (2013)

Quignon, F., Kiéné, L., Lévi, Y., Sardin, M., Schwartzbrod, L.: Virus behaviour within a distribution system. Water Sci. Technol. **35**, 311 (1997)

Storey, M.V., Ashbolt, N.J.: Persistence of two model enteric viruses (B40-8 and MS-2 bacteriophages) in water distribution pipe biofilms. Water Sci. Technol. **43**, 133 (2001)

Tars, K., Bundule, M., Fridborg, K., Liljas, L.: The crystal structure of bacteriophage GA and a comparison of bacteriophages belonging to the major groups of *Escherichia coli* leviviruses. J. Mol. Biol. **271**, 759 (1997)

Tatchou-Nyamsi-König, J.A., Dague, E., Mullet, M., Duval, J.F.L., Gaboriaud, F., Block, J.-C.: Adhesion of *Campylobacter jejuni* and *Mycobacterium avium* on polyethylene terephtalate (PET) used for bottled waters. Water Res. **42**, 4751 (2008)

Wingender, J., Flemming, H.-C.: Biofilms in drinking water and their role as reservoir for pathogens. Int. J. Hyg. Environ. Health **214**, 417 (2011)

Woody, M.A., Cliver, D.O.: Effects of temperature and host cell growth phase on replication of F-specific RNA coliphage Qβ. Appl. Environ. Microbiol. **61**, 1520 (1995)